SYNTHETIC BIOLOGY

Engineering off the beaten pathway

Interfacing synthetic diverters with a native yeast signaling pathway adds a robust layer of cell fate control.

Cells decide on weighty matters by processing information from their environment through signaling pathways. Broken pathways or spoiled environments can lead to poor decisions or disease, and a key goal in synthetic biology is to be able to control these decisions by changing the flow of information.

The mitogen-activated protein kinase signaling pathway has been a favorite engineering target. In yeast, it halts division and prepares the organism for mating in response to a pheromone. Previous modifications to this pathway, such as engineered feedback loops and repurposed signaling scaffolds, have required the alteration of endogenous genes. Many of these surgeries are successful but have the side effect of changing the yeast's character: the microbe no longer behaves like itself in its natural environment.

Christina Smolke at Stanford University and her colleagues asked whether it was possible to wrest control of signaling in a seamless way that preserves wild-type behavior while making yeast responsive to artificial signals. "We wanted to look at whether we could build control systems that interface with native signaling pathways so that we weren't reconstructing everything from scratch," Smolke says.

The first challenge was to identify pathway control points. "The cell can have a lot of endogenous control systems which modify levels of pathway regulators," says Smolke. "You might not be able to see any effect on signaling." The team systematically titrated levels of various components such as kinases, finding only one regulator that enhanced signaling and one that inhibited pathway activation when overexpressed. Putting these under control of a pathway-responsive promoter enhanced the effect of the positive regulator at lower concentrations and attenuated the degree of inhibition that the negative regulator could achieve.

To bring these elements under human control, the researchers placed an RNA-based transducer at the end of each transcript. The transducers were developed in previous work from the Smolke lab; they translate levels of small-molecule inducers such as tetracycline or theophylline into RNA levels and, ultimately, protein levels. A sensing domain binds the inducer and inhibits an RNA-cutting ribozyme as inducer concentration increases, thus stabilizing the transcript to which they are both fused.

The group termed the promoter, regulator and RNA transducer "molecular network diverters." Their modular design allows easy swapping of promoters to put the regulators under different levels of constant or feedback control and the switching out of transducers tuned to respond to different levels of various small molecules.

Positive and negative diverters performed well in directing the pathway, but the biggest challenge—and the greatest insights—came from incorporating the push and pull of both diverters into a single "dual diverter" that directs one of three possible fates: native response to pheromone, and mating or not mating in response to different inducers.

"The two different diverters can antagonize each other," says Smolke. "And what we mean by that—and this is true of a lot of biological systems—is that there's a low level of activity from either of the diverters in their off state." The leaky activity was not enough to direct fate on its own, but it impinged on the switching activity of the other diverter. Engineering the dual diverter required stepping back and modeling what to expect.

A simple black-box model showed that additional 'booster' elements could overcome the effects of the second regulator being present. Modeling "shrinks the design space," says Smolke, and it helped the group design diverters that trigger robustly in their 'on' state to cause a mating fate.

The team's previous work developing individual parts was critical to this recent success. "It was important that we had those components in place so that we could test the design space and readily arrive at a solution," says Smolke.

The approach has great potential. The group is working on building control systems that interface with native signaling pathways in human cells, with the possibility of eventually sensing and responding to disease states, leading to cell therapies.

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RESEARCH PAPERS
Galloway, K.E. *et al.* Dynamically reshaping signaling networks to program cell fate via genetic controllers. *Science* doi:10.1126/science.1235005 (15 August 2013).